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(57) Abstract

The invention relates to the use as a contrast agent in MR imaging of a substance which has a first and a second relaxivity state, which has relaxivities differing by a factor of at least 5 in said first and second states, and which is convertible *in vivo* from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second electronic state does or does not occur.

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NMR IMAGE COMPOUNDS

This invention relates to compounds useful as contrast agents in magnetic resonance imaging and to methods of imaging using such compounds.

Magnetic resonance (MR) imaging is a well established imaging modality in which the image is derived from the intensity of the nmr signal from protons (usually water protons) in the subject under study. Because most tissue has an approximately 80% water content, contrast in MR imaging is attained by the application of pulse sequences that reveal differences in the relaxation times (T_1 and T_2) of the tissues. As with other diagnostic imaging modalities such as CT and ultrasound, contrast agents may be used in MR imaging procedures to enhance contrast in the images produced, eg. to allow clearer differentiation between different tissue types or between healthy and non-healthy tissue. In MR imaging, the contrast agents conventionally are chelated paramagnetic species (eg. Gd DTPA, Gd DTPA-BMA and Gd HP-DO3A, available commercially under the trade names Magnevist, Omniscan and Pro-Hance), which achieve contrast enhancement because of their relaxivities, their ability to decrease the relaxation times of water protons.

A proposal has been made, in WO 96/38184, that "triggered" paramagnetic metal ion complexes be used as MR contrast agents. As described in WO96/38184, the trigger mechanism has the paramagnetic complex being "turned on" as an MR contrast agent by the presence of a target substance which interacts with the agent complexing the paramagnetic metal ion so as to free an inner sphere coordination site and allow water molecule exchange to take place at the freed-up site. In the absence of the target substance, the complexed paramagnetic metal ion has no inner sphere coordination sites available for water molecule exchange and in this

state the contrast agent is considered to be turned off.

This concept of a triggered MR contrast agent however has a major defect which will hinder practical application of the concept. Thus in the "turned off" state the complex will still function fairly effectively as an MR contrast agent since both inner-sphere and outer-sphere water coordination contributes to the agent's relaxivity. The inventors of WO96/38184 indirectly acknowledge this drawback when they refer to the degree of change in MR signal that is sufficient to be detectable in the image as being as low as 2 to 5%, well below the conventionally accepted threshold of 10% (see for example Chem. Rev. 87: 901-927 (1987)). The relaxivity of the gadolinium chelates of WO96/38184 will be reduced by about one half (but not eliminated) if inner sphere coordination of water is prevented. Thus the triggered agents of WO96/38184 are not so much switched off as dimmed by about half by the absence of the target substance. Accordingly the selectivity and sensitivity desired by the authors is not possible due to the unavoidable outer-sphere contribution.

It has now been realised that triggered MR imaging of contrast agents may be achieved significantly more efficiently by using the "target substance" to change the contrast agent between states in which the relaxivity (r_1) differs by a factor of 5 or more, preferably 10 or more, eg. 5 to 20 or even higher (eg. up to 100).

This can be achieved either by switching to a lower relaxivity state with little or no relaxivity or alternatively by switching on/off an inner sphere deriving relaxivity which is significantly higher than (eg. 5 times or greater than) the outer sphere deriving component of the relaxivity. This can be achieved in a number of ways as described below.

Thus viewed from one aspect the invention provides a method of generating a contrast enhanced image of a human or non-human (preferably mammalian) animal subject

which comprises administering to said subject an effective amount of a magnetic resonance imaging contrast agent and generating a magnetic resonance image of at least a part of said subject containing said agent, the improvement comprising using as said agent a substance which has a first and a second (r_1) relaxivity state, which differs in relaxivities in said first and second states by a factor of at least 5, preferably at least 10, eg. up to 20 or even up to 100, and which is convertible in vivo from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

One way in which to change between high and low r_1 relaxivity states for the contrast agent is to change the electronic state of the complexed metal in a contrast agent between higher and lower relaxivity states. In this regard the means for effecting the change may be localised normal or abnormal biological activity, an administered chemical agent or an applied physical means (eg. illumination with light). At its simplest, the change in electronic state might be effected as a change in oxidation state of the complexed metal ion, eg. from a paramagnetic to a diamagnetic state, from a diamagnetic to a paramagnetic state, or from one paramagnetic state to another, eg. from a non-spherically symmetric electronic ground state to a spherically symmetric electronic ground state, or a change from a non-spherically symmetric electronic ground state to a spherically symmetric excited state. The non-spherically symmetric state will have a much lower associated relaxivity than the spherically symmetric state and accordingly the contrast difference between the "on" and "off" states of the switchable agent is large.

The conversion from first to second electronic state may for example be as a result of a redox reaction, for example conversion of a chelated metal ion from one oxidation state to another (one or both of

which are paramagnetic), or as a result of stimulated electronic transition between a high-relaxivity state and a low-relaxivity state where the lower relaxivity results from lower electronic symmetry (eg. between the high relaxivity state of tetrahedral Co(II) complexes (where the three unpaired d electrons are in a t_{2g} state) and a low relaxivity state (where two of the unpaired d electrons are in a t_{2g} state and the third is in the e_g state)), or as a result of a change of or in a ligand complexing a paramagnetic metal ion, or as a result of quenching of a persistent free radical contrast agent, or as a result of electromagnetic radiation stimulation of a paramagnetic contrast agent from one electronic state to another having a different spin quantum number (eg. in a fluorescence or phosphorescence excitation).

For the tetrahedral Co(II) complexes mentioned above, in the high relaxivity state, the three d electrons in the t_{2g} state make up a state that is cubically symmetric - a degree of symmetry sufficiently close to spherical symmetry for the purposes of the invention. With two unpaired electrons in the t_{2g} state and one in the e_g state, the overall symmetry is significantly removed from spherical and relaxivity is lower. The transition involved need not involve a change in spin quantum number.

One example of on/off switching by a redox reaction occurs in the case of atoms with four unpaired d electrons with a square-planar symmetry ligand field with high spin. In this case either oxidation or reduction will bring about a decrease in relaxivity (the oxidation effect being greater than the reduction effect) even though the starting state has a non-spherically symmetrical electronic ground state. An example of such a system is the class of Mn(III) porphyrins, compounds which have high relaxivities. In these compounds, the anisotropy of the electronic wave function due to its symmetry (which includes the fact that the inner-sphere water molecules are located along

the D4 rotation axis) increases the dipolar interaction between the Mn(III) ion and the protons of the water molecules bound to it. Oxidation to Mn(IV) causes a substantial loss of relaxivity due to the loss of an unpaired electron - the Mn(IV) has only three unpaired d electrons which lowers the magnetic moment and destroys the favourable anisotropy. Reduction to Mn(II) likewise causes a drop in relaxivity, but by a smaller amount.

An alternative way to switch between high and low r_1 relaxivity states is to have as the high relaxivity state a very high relaxivity compound (such as the polymeric chelates of PCT/GB96/01308, a rigid paramagnetic polychelate, or a large substrate-bound paramagnetic chelate, eg. a vector targeted lanthanide chelate or polychelate) and to have as the low relaxivity compound the same material (i) with water-coordination sites reversibly blocked by an enzymically removable blocking group (as in WO96/38184) and/or (ii) with a targeting vector (eg. an antibody, antibody fragment, or an oligopeptide binding motif (such as RGD)) which in that state is not bound to its intended substrate.

Thus the switching between low and high relaxivity states may be achieved by binding of the targeting vector or displacement of the blocking groups or alternatively by interaction at the target site to change a high relaxivity conformation into a low relaxivity conformation, or vice versa. Such conformational changes can be achieved for the compounds of PCT/GB96/01308 by changing the chemical nature of their immediate environment (eg. by the presence or addition of urea).

Another way to switch between high and low r_1 relaxivity states is to have as the high relaxivity state a very high relaxivity compound at a non-neutral pH and to have as the low relaxivity compound the same material at substantially neutral pH. Thus, switching between low and high relaxivity states may be effected

by an increase or decrease in pH at the target site, e.g. as a result of a biological process. Complexes particularly suitable for use in this aspect of the invention include Fe(III) complexes.

The high-spin oxidation state of Fe(III) (S=5/2) gives it the potential of being a relatively good relaxation agent. However, its relaxivity can be much lower than expected due to several reasons (see e.g. S.H. Koenig et al., "Relaxometry of Paramagnetic Ions in Tissue", in Metal Ions in Biological Systems, Helmut Sigel, Ed., Vol. 21, Applications of Nuclear Magnetic Resonance to Paramagnetic Species, Marcel Dekker, Inc. New York, 229-270, 1987). One reason is a slow exchange of water molecules between the inner coordination sphere of the Fe(III) ion and the bulk, even when the Fe(III) ion is chelated leaving at least one open site for an exchangeable water molecule to bind. Typically, the exchange of water molecules between the inner coordination sphere and the bulk is slow. For a high proton relaxivity, all that is necessary is that the *protons* of water molecules exchange between the inner coordination sphere and the bulk. At physiological pH, this proton exchange is too slow to allow for a high relaxivity. However, at lower pH values, the proton exchange becomes acid catalyzed, the proton exchange rate, and hence the relaxivity, increases. Similarly, at higher pH values, the proton exchange becomes base catalyzed, the proton exchange rate, and hence the relaxivity, increases. Consequently, there is an "on-off" switch for MR imaging. At neutral pH, the relaxivity is low, and the image intensity is low. At pH values lower or higher than neutrality, the relaxivity is higher, and the image intensity is higher.

The association of individual molecules, e.g. porphyrin complexes, can also be affected by pH. This effect has been shown in relation to Fe(III) porphyrins (see S.H. Koenig et al., Magnetic Resonance in Medicine 4: 252-260, 1987). When associated, the relaxivity of

such compounds may decrease, either due to an exclusion of water molecules from the inner coordination sphere of the Fe(III) ion, or due to magnetic interactions between the adjacent Fe(III) ions, similar to that which occurs when Mn(III) porphyrins become agglomerated (see K.E. Kellar et al., Inorganic Chemistry 31: 1353-1359, 1992). Since association is likely to occur at higher pH values, so again there is an "on-off" switch with relaxivity which is sensitive to pH.

Preferably, the contrast agent, e.g. an Fe(III) chelate, for use in accordance with this aspect of the invention may be further conjugated to a macromolecule. In this way, the relaxivity of the contrast agent is further increased thereby enhancing the sensitivity of "on-off" switch between relaxivity states.

The means by which conversion from one state to another may be achieved may be a biological process or malfunction, eg. resulting from the presence or absence of oxygen or oxidation or reduction promoting agents or of natural free radicals or free radical scavengers. Alternatively the means for conversion may be a chemical agent administered to the subject, eg. a radical scavenger or redox reagent capable of delivery to or accumulation at a desired target site within the body, or designed for release at such a site for example a tumour or oedema. In some cases, activation of the agent may involve application of light, preferably with a wavelength of from 600 to 1300 nm in order to minimise absorption by the body.

Thus by way of example the agent could be an iron or manganese compound, preferably a chelate complex, which can be switched between the II and III oxidation states by biological activity or by redox reagents. Fe(III) and Mn(II) have spherically symmetric states while Fe(II) and Mn(III) have non spherically symmetric electronic states. While both the II and III states are paramagnetic, the spherically symmetric states have much higher relaxivities, except in the case of the Mn(III)

porphyrins discussed above.

As mentioned above, in one aspect of the invention the relaxivity of the contrast agent may be switched as a result of a change in pH. Contast agents for use in the method of the invention may thus be used to detect areas of the body which are acidic or basic due to physiological or disease processes. Typically, they may be used to detect regions of pH of about 4 to ~5.5 within the body by appropriate selection as the contrast agent of a substance having a pKa value above or below a predictable threshold.

For example, many tumors exhibit a lower extracellular pH, e.g. as low as 5.5, typically between ~5.5 and ~7.7. This is a result of decreased vascular perfusion resulting in chronic hypoxia and increased lactic acid levels, exacerbated by the typically higher metabolic rate of cancerous cells. On the other hand, necrotic areas within tumors may exhibit a higher, more basic, pH. Acidic tumor types which may be detected using the method of the invention include malignant melanoma, squamous cell carcinoma, sarcomas and adenocarcinomas (see Thistlewaite et al., Int. J. Radiation Oncology Biol. Phys. 11: 1647-1652, 1985).

Osteoporosis is a degenerative bone disorder. During the physiological process of bone resorption osteoclasts excavate small pits throughout the bone, creating a zone of reduced pH between the osteoclast and the bone tissue. pH values as low as 4.0 have been measured in the active erosion zones (see Silver et al., Cell Res. 175: 266-267, 1988). Effective imaging of this erosion zone using the method of the invention may be used to provide vital information regarding the effectiveness of therapies used in the treatment of osteoporosis. In this way, the clinician may readily determine the therapeutic effect of a given drug and use the information either to continue therapy or to change therapies.

Measurement of local osteoclastic activity using

the method of the invention may also be used to evaluate other bone remodelling activities such as the repair of fractures, the treatment of Paget's disease or to evaluate the extent of expanding lesions in bone, such as tumors, in which resorption may take place at the bone surface in contact with the lesion.

In the method of the invention, the region in which the conversion from first to second state occurs will preferably be identified, eg. by comparison with a "native" image in the collection of which the means for conversion has not been administered or activated or with a comparison body site in which the biological process responsible for the conversion does not occur.

The contrast agent used in the method of the invention may be for example a complex of a metal ion which is in or may be converted into a paramagnetic state (eg. one which is capable of redox conversion between two paramagnetic states or between a paramagnetic and a diamagnetic state). Suitable candidates may be found in the transition metals, the lanthanides and the actinides, especially Mn and Fe. In this event the complexing agent will suitably be one which presents the metal in a biotolerable form, eg. a polyaminopolyacid chelating agent of the type well known for MR agents and radiopharmaceuticals, for example DTPA, EDTA, DTPA-BMA, D03A, DOTA, HP-DO3A, TMT, DPDP, etc. In this regard the reader is referred to the patent publications of metal chelates from Schering, Nycomed, Salutar, Bracco, Mallinckrodt, Guerbet, Sterling Winthrop, etc. Examples include US-A-4647447, US-A-5362475, US-A-5534241, US-A-5358704, US-A-5198208, US-A-4963344, EP-A-230893, EP-A-130934, EP-A-606683, EP-A-438206, EP-A-434345, WO 97/00087, WO 96/40274, WO 96/30377, WO 96/28420, WO 96/16678, WO 96/11023, WO 95/32741, WO 95/27705, WO 95/26754, WO 95/28967, WO 95/28392, WO 95/24225, WO 95/17920, WO 95/15319, WO 95/09848, WO 94/27644, WO 94/22368, WO 94/08624, WO 93/16375, WO 93/06868, WO 92/11232, WO 92/09884,

WO 92/08707, WO 91/15467, WO 91/10669, WO 91/10645, WO 91/07191, WO 91/05762, WO 90/12050, WO 90/03804, WO 89/00052, WO 89/00557, WO 88/01178, WO 86/02841 and WO 86/02005.

Additionally, the agent may be a high relaxivity agent such as described in PCT/GB96/01308, with water coordination sites blocked by displaceable blocking moieties as described in WO96/38184, or any other relatively rigidly structured paramagnetic polychelate, eg. a dendrimer according to WO 93/06868 with a short linkage or a hydrophobic linkage between dendrimeric branching sites.

Such chelating agents may if desired be conjugated to biological vectors so as to target actively or passively to the desired regions of the body. Conjugation of metal chelates to targeting vectors is discussed for example in WO-93/21957 and US-A-5595725 (Schering).

Where the targeting vector is such as to bind the agent to a target site, relaxivity will be increased as a result and in one embodiment of the invention the triggering of enhanced relaxivity may be achieved by a combination of the freeing up of a coordination site according to WO96/38184 and binding to a larger structure, eg. a cell wall or the wall of a body duct using a vector (eg. an antibody, antibody fragment, or an oligopeptide binding motif (such as RGD) conjugated to a compound according to WO96/38184.

The contrast agent for use in the method of the invention may be for example a complex of a metal ion having at least one open coordination site for the exchange of water molecules. Such agents are capable of switching between first and second relaxivity states as a result of a change in pH. Suitable complexing agents include macrocyclic chelants having an open coordination site for water, e.g. porphyrin-like molecules and the pentaaza macrocyclic ligands of Zhang et al (Inorg. Chem. 37(5):956-963, 1998), phthalocyanines, crown

ethers e.g. nitrogen crown ethers such as the sepulchrates, cryptates etc., hemin (protoporphyrin IX chloride) and heme (available from Porphyrin Products, Inc. of Logan, Utah, USA) and chelants having a square-planar symmetry. Alternatively, the complexing agent may comprise a polyacid ligand capable of protonating a coordinating group thereby freeing up a coordination site for water molecules at a particular pH. Particularly suitable for use in the method of the invention are chelate complexes of iron, especially iron(III). Appropriate iron(III) complexes can be identified using the method of Rustad et al as outlined in J. Chem. Physics 102(1):427-431, 1995).

Such chelating agents may if desired be bound to a macromolecule, if necessary to enhance the sensitivity of the "on-off" switch between relaxivity states. Examples of suitable macromolecules include proteins and polymers, e.g. that prepared in accordance with Example 2 of WO98/10797, and macrostructures such as liposomes in which the chelate is bound to the outer surface.

For administration into the GI tract, it may be unnecessary to chelate the metal and thus for this route simple salts, eg. Mn or Fe chlorides, may be used.

Alternatively the contrast agent may be a free radical, eg. one as discussed in EP-A-133674 (Schering) or US-A-5530140 (Nycomed). In this case the "on/off switch" for the contrast agent is provided by naturally occurring or administered materials which scavenge or quench the free radical destroying its paramagnetism and hence switching off from high relaxivity to very low or no relaxivity.

The contrast agent may be administered by any convenient route, eg. topical, transdermal, nasal, sub-lingual, oral, rectal, by direct instillation into an externally voiding body cavity (eg. lungs, uterus, GI tract and bladder), or subcutaneously, intramuscularly, interstitially or into the vasculature, eg. by injection or infusion. In general administration into the

vasculature or into the GI tract will be preferred routes.

For administration, the contrast agent may be formulated together with appropriate conventional pharmaceutically acceptable carrier or excipients, such as liquid carriers (eg. saline or water for injections), pH and osmolality regulators, stabilizers, viscosity modifiers, surfactants, bulking agents, skin penetration agents, flavourings, solid or semi-solid carriers (eg. hydrophilic gels), aerosol dispersants, etc.

The dose of contrast agent required will depend on the species and condition under study, the selected contrast agent, and the administration route. However in general doses for iv administration will normally be in the range 0.001 to 5.0 mmol paramagnetic centre/kg bodyweight (where by paramagnetic centre is meant a metal atom which is or becomes paramagnetic, or a free radical electron).

If a chemical agent (a "trigger", for example an enzyme, a redox agent or a free radical scavenger) is administered to trigger the conversion between states of different relaxivity, then this can be administered together with the contrast agent or separately, eg. before or after or even simultaneously in the event that the administration site is different. If coadministered, then the trigger may be formulated to contact the contrast agent only after delivery or on reaching the target site. Thus for example it may be encapsulated in a matrix or membrane (eg. a vesicle membrane) which breaks down or is broken down at the desired target site. If the method of the invention is used intraoperatively, eg. to highlight damage to a particular tissue or organ or to delineate a mass to be removed, the trigger may be applied to the operating site during the operation so as to switch on the contrast agent at the cutting site. In this event the trigger may be a chemical agent as discussed above, the air, or an applied physical stimulus.

The compositions containing both the contrast agent and a chemical trigger are themselves new and form a further aspect of the invention. Viewed from this aspect the invention provides an MR contrast agent composition comprising as an MR contrast agent a physiologically tolerable substance, which has a first and a second relaxivity state, which has relaxivities differing by a factor of at least 5 in said first and second electronic states, and which is convertible in vivo from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, together with an optionally encapsulated physiologically tolerable trigger substance capable of converting said contrast agent between said first and second states.

Viewed from a further aspect the invention also provides the use of a physiologically tolerable MR contrast agent substance which has a first and a second relaxivity state, which has relaxivities differing by a factor of at least 5 in said first and second states, and which is convertible in vivo from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, for the manufacture of a diagnostic contrast medium for use in a method of diagnosis involving image generation according to the method of the invention.

All documents referred to herein are hereby incorporated by reference.

The invention will now be described further with reference to the accompanying non-limiting Examples.

EXAMPLE 1

The hematoporphyrin derivative HPD is known to be selectively retained by cancerous tumours. Irradiation of the retained HPD with light at one of the absorption maxima of the dye then results in death of the tumour cells. It is known that both singlet oxygen and hydroxyl radicals are involved in microsomal damage (see Das et al. "Role of Active Oxygen Species in the Photodestruction of Microsomal Cytochrome P-450 and Associated Monooxygenases by Hematoporphyrin Derivative in Rats," Cancer Res. (1985) 45, 608). The production of hydroxyl radicals may involve the Fenton reaction.

$$H_2O_2 + Fe^{2+} \rightarrow OH^{\cdot} + OH^{-} + Fe^{3+}$$

HPB is i.p. injected at a concentration of 5 mg/kg body weight into Wistar rats on which a tumour has been induced on the outside of one of the hind legs 24 hours prior to photodynamic therapy. A solution of 0.1 M ferrous chloride is injected i.v. 15 minutes prior to therapy. The irradiation of the tumour is done with light at 630 nm emitted by a dye-pumped, argon ion laser. The light results both in cell destruction and the generation of ferric ions that act as MRI relaxation reagents. MRI is then used to monitor the course of the photodynamic therapy as cell destruction proceeds.

Claims:

1. A method of generating a contrast enhanced image of a human or non-human animal subject which comprises administering to said subject an effective amount of a magnetic resonance imaging contrast agent and generating a magnetic resonance image of at least a part of said subject containing said agent, the improvement comprising using as said agent a substance which has a first and a second relaxivity state, which has relaxivities differing by a factor of at least 5 in said first and second states, and which is convertible *in vivo* from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second relaxivity state does or does not occur.

2. A method as claimed in claim 1 wherein said agent comprises a substance having first and second relaxivity states which differ in relaxivity by a factor of at least 10.

3. A method as claimed in claim 1 or claim 2 wherein said agent comprises a substance having first and second relaxivity states which differ in relaxivity by a factor of up to 20.

4. A method as claimed in claim 1 or claim 2 wherein said agent comprises a substance having first and second relaxivity states which differ in relaxivity by a factor of up to 100.

5. A method as claimed in any one of claims 1 to 4 wherein said agent comprises a chelate complex of a metal ion, or physiologically tolerable salt thereof, the change between said first and said second relaxivity states being effected by a change in the electronic state of the complexed metal ion.

6. A method as claimed in claim 5 wherein said change in electronic state is effected as a change in oxidation state of the complexed metal ion.

7. A method as claimed in claim 6 wherein said change in oxidation state is effected as a change from a paramagnetic to a diamagnetic state, as a change from a diamagnetic to a paramagnetic state, or as a change between two paramagnetic states of the complexed metal ion.

8. A method as claimed in claim 7 wherein said change between two paramagnetic states is effected either as a change from a non-spherically symmetric electronic ground state to a spherically symmetric electronic ground state, or as a change from a non-spherically symmetric electronic ground state to a spherically symmetric electronic excited state.

9. A method as claimed in any one of claims 5 to 8 wherein said agent comprises a physiologically tolerable complex of a transition metal, lanthanide metal or actinide metal ion, or a physiologically tolerable salt of such a chelate.

10. A method as claimed in claim 9 wherein said agent is a chelate complex of a paramagnetic ion of iron or manganese, or a physiologically tolerable salt thereof.

11. A method as claimed in any one of claims 5 to 10 wherein said chelate complex is a complex of a chelant selected from the group consisting of DTPA, EDTA, DTPA-BMA, DO3A, DOTA, HP-DO3A, TMT and DPDP.

12. A method as claimed in any preceding claim wherein said agent comprises a substance having water coordination sites reversibly blocked by a removable blocking group.

13. A method as claimed in any preceding claim wherein said agent is conjugated to a biological vector capable of targeting said agent to a desired region within the body.

14. A method as claimed in claim 13 wherein said biological vector is selected from the group consisting of an antibody, an antibody fragment and an oligopeptide binding motif.

15. A method as claimed in any one of claims 1 to 4 wherein said agent comprises a chelate complex of a metal ion having at least one coordination site capable of the exchange of water, or a physiologically tolerable salt thereof.

16. A method as claimed in claim 15 wherein said agent comprises a physiologically tolerable complex of iron, or a physiologically tolerable salt of such a chelate.

17. A method as claimed in claim 15 or claim 16 wherein said chelate complex is a complex of a chelant selected from the group consisting of porphyrins and porphyrin-like molecules, phthalocyanines, crown ethers and chelants having a square planar symmetry.

18. A method as claimed in any preceding claim wherein conversion between said first and second relaxivity states is effected *in vivo* by a localised normal or abnormal biological process, by an administered chemical agent or by illumination of said agent with light.

19. A method as claimed in claim 18 wherein conversion between said first and second relaxivity states is effected *in vivo* by the presence or absence of oxygen or oxidation or reduction promoting agents or of natural free radicals or free radical scavengers.

20. A method as claimed in claim 18 wherein said chemical agent is a radical scavenger or redox reagent.

21. A method as claimed in claim 18 wherein conversion between said first and second relaxivity states is effected by illumination with light having a wavelength of from 600 to 1300 nm.

22. An MR contrast agent composition comprising a physiologically tolerable substance, which has a first and a second relaxivity state, which has relaxivities differing by a factor of at least 5 in said first and second states, and which is convertible *in vivo* from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second electronic state does or does not occur, together with an optionally encapsulated physiologically tolerable trigger substance capable of converting said contrast agent between said first and second electronic states.

23. A composition as claimed in claim 22 wherein said trigger substance is an enzyme, a redox agent or a free radical scavenger.

24. The use of a physiologically tolerable MR contrast agent substance which has a first and a second relaxivity state, which has relaxivities differing by a factor of at least 5 in said first and second states, and which is convertible *in vivo* from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second electronic state does or does not occur, for the manufacture of a diagnostic contrast medium for use in a method of diagnosis involving image generation according to the method of the invention.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01173

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 38184 A (CALIFORNIA INST OF TECHN ;MEADE THOMAS (US); FRASER SCOTT (US); JA) 5 December 1996 cited in the application see page 12, line 24 - page 16, line 3; claims 1-16	1-24
Y	EP 0 438 206 A (SCHERING AG) 24 July 1991 see page 14, line 38 - page 15, column 3 see page 17, line 13 - line 19 see claims 1-16	1-24
Y	WO 96 40274 A (NYCOMED IMAGING AS ;COCKBAIN JULIAN (GB)) 19 December 1996 cited in the application see page 14 - page 15	1-24

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 1998	28. 08. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seegert, K

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01173

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 21845 A (UNIV TEXAS ;PHARMACYCLICS INC (US); SESSLER JONATHAN L (US); MODY) 17 August 1995 see page 21, line 29 - page 22, line 17 see page 26, line 4 - line 35 see claims 1-17	1-24
Y	US 4 877 872 A (MORGAN ALAN R ET AL) 31 October 1989 see column 6, line 25 - line 35; claims 1-3	1-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/01173

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

In view of the large number of compounds which are only functionally defined in the independent claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, Chapter III, paragraph 2.3).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/01173

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